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PRINCIPAL INVESTIGATOR: Adrian V. Lee, Ph.D.

CONTRACTING ORGANIZATION: University of Pittsburgh
Pittsburgh, PA 15213-3320

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14. ABSTRACT

The genetic basis of cancer has been firmly established in the last few decades. Genomic instability is a hallmark feature of virtually all breast cancer cells, and is caused either by inherited mutations in genes that control genomic fidelity and stability (particularly in DNA repair pathways), or somatic mutations that are acquired during breast cancer progression. We originally identified translocations in 3 DNA repair genes (RAD51C, EYA2, BRIP1) in MCF7 breast cancer cells, and hypothesized that structural genomic alterations in genes that are themselves actually involved in DNA repair enhance the level of genomic instability and ultimately affect breast cancer progression and prognosis. However, we found that these translocations are specific to MCF7 cells (private mutations) and not present in any other cell lines. In addition, the RAD51C-ATXN7 fusion gene alternatively spliced and not expressed as a full length fusion gene. The short isoform wasn't found to have altered biochemical function. In summary, this study supports other recent reports in finding that DNA translocations aren't recurrent in human breast cancer.

15. SUBJECT TERMS
Breast Cancer, DNA repair, RAD51C, EYA2, BRIP1, translocation

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1) Introduction

The genetic basis of cancer has been firmly established in the last few decades. Genomic instability is a hallmark feature of virtually all breast cancer cells, and is caused either by inherited mutations in genes that control genomic fidelity and stability (particularly in DNA repair pathways), or somatic mutations that are acquired during breast cancer progression. The importance of DNA repair in breast cancer is highlighted by the fact that inherited breast cancer is associated with germline mutations in ten different genes associated with genome stability and fidelity. Importantly, the central role of DNA double stranded break repair (DSBR) in both hereditary and sporadic breast cancer may provide an Achilles heel that can be targeted therapeutically. Thus, defects in DSBR pathways lead cells to become hypersensitive to DNA damaging agents such as mitomycin C or cisplatin. Using paired end sequencing, we generated a map of breaks in genomic DNA in a breast cancer cell line named MCF-7 (1). This study gave us a unique insight into the genomic instability in MCF-7 cells and showed that a number of genes that had undergone structural change (translocation, deletion, or inversion) were tumor suppressor genes and were mostly repaired by non-homologous end joining an error-prone method of DNA double strand break repair. Intriguingly, we identified translocation of three genes, *RAD51C*, *BRIP1,* and *EYA2*, all of which are all central to DSBR, leading to the novel and exciting IDEA that genes important for genomic integrity and homologous recombination are themselves structurally altered at the genomic level and thus potentially non-functional.

We hypothesized that structural genomic alterations in the genes that are actually themselves involved in DNA repair enhance the level of genomic instability and ultimately affect breast cancer progression and prognosis. We hypothesized that alterations in *BRIP1*, *RAD51C, and EYA2* would render cell hypersensitive to DNA damaging agents and that fidelity of the DSBR pathway, measured at the genomic level, might be a candidate biomarker for personalizing therapy.

SPECIFIC AIMS

1) Determine the prevalence of recurrent and selected aberrations in BRIP1, RAD51C, and EYA2 in a large cohort of human breast tumors, and correlate presence with prognosis and/or response to therapy.

2) Test whether truncations or fusions of BRIP1, RAD51C and EYA2 result in loss of function or dominant negative effects on DNA repair, sensitization to DNA damaging agents, and if the loss of these proteins contributes to genomic instability.

2) Body

Aim 1

Task 1) Analyze BRIP1, RAD51C, and EYA2 protein in 20 breast cancer cells lines by immunoblot (months1-4)

We started Aim 1 Task 1 and Aim 1 Task 2 at the same time. Measurement of BRIP1, RAD51C, and EYA2 by RT-PCR was successful as noted in the next Task (Task 2). However, measurement of protein expression was more difficult due to a lack of suitable antibodies. We immunblotted for RAD51C and BRIP1 multiple times with several results indicating that we may have detected RAD51C fusion protein expression in several cell lines (as noted in the previous report), however, based upon further analysis we believe that this immunoblotted protein was indeed an artifact, as further data (detailed later) suggest that the translocation and fusion gene only exist in MCF-7 cells. This is one of the concerns with immunoblotting and the reliance on suitable specific antibodies. We continued to test antibodies, however, as the project continued and it became clear that the fusion genes we were studying were only expressed in a single cell line, were sometimes amplified (BRIP1), and didn't have an obvious function, we then stopped attempting this aim.

Task 2) Measure BRIP1, RAD51C, and EYA2 mRNA by RT-PCR (months 1-4).

We measured mRNA expression of RAD51C, BRIP1 and EYA2 by Q-RT-PCR on a panel of 32 breast cancer cell lines and used MCF10A (immortalized but non-transformed) and normal female breast RNA (purchased from Life Technologies) as a control (Figure 1). Our hypothesis was that RAD51C expression would be elevated in MCF-7 cells as it is found as an expressed fusion mRNA (RAD51C:ATXN7) and indeed we found this to be the case (Figure 1 top panel).

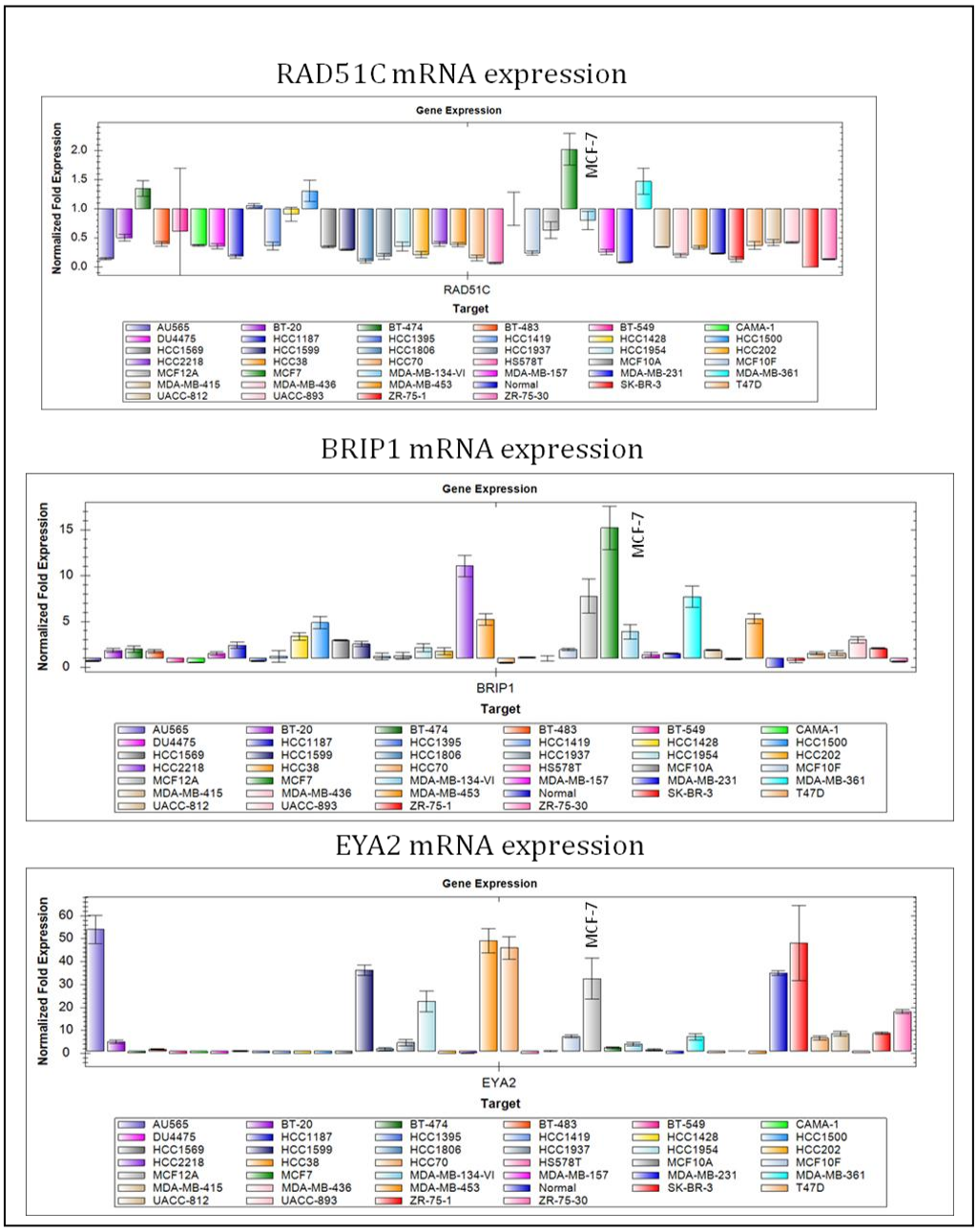

Figure 1: mRNA expression of RAD51C, BRIP1, and EYA2 in a panel of 32 breast cancer cell lines. RNA was extracted from a panel of 32 breast cancer cell lines and MCF10A immortalized but non-transformed cells. mRNA levels for RAD51C, BRIP1 and EYA2 were measured by Q-RT-PCR using β-actin as a normalization control. Normal represented normal female RNA purchased from Life Technologies.

MCF7 cells showed the highest level of RAD51C mRNA, however, high expression of RAD51C was limited to this cell line only. All other cells had low levels of RAD51C (Figure 1). For BRIP1 and EYA2 the translocation we originally reported (1) resulted in truncation of the gene with the final exons being replaced by non-genic DNA. As this eliminates the mRNA polyA tail, we believed this would result in an unstable mRNA that is rapidly degraded, thus resulting in reduced mRNA levels. Cells with this translocation would thus have one mutant allele and thus would be expected to have either half the level of mRNA compared to normal cells, or if the other allele is also genomically rearranged or mutated they might have no transcript at all. We thus hypothesized that MCF7 cells would have low levels of expression compared to other cell lines, and that breast cancer cells would be lower than MCF10A and normal RNA. However, for both BRIP1 and EYA2 we found that MCF7 had the highest level of expression and that most cell lines had expression that was higher than MCF10A and normal RNA. This result didn't fit with our hypothesis of these genes being mutated with reduced expression (e.g. tumor suppressor genes) in breast cancer. Despite this negative result we continued to examine the expression of specific RAD51C:ATXN7 fusion genes, and allele specific expression of BRIP1 and EYA2 to see if this might explain their role in more detail.

As RAD51C is expressed as a fusion gene with ATXN7 we specifically set out to examine if the levels of this fusion gene and whether it existed in any other cell lines. We first performed RT-PCR of RAD51C-ATXN7 to examine and clone mRNA isoforms. We isolated the original splice isoform we reported in our original manuscript (1) which spliced exon 7 of Rad51C to ATXN7. This resulted in an early translation stop codon and a truncated RAD51C protein (Figure 2A - schematic; 2B – sequence). However, we also cloned a different RNA splice variant which spliced exon 6 of RAD51C to exon 6 of ATXN7 and resulted in a complete in frame transcript (Figure 2A - schematic; 2C – sequence).

Figure 2: Identification of short and full-length RAD51C-ATXN7 fusion mRNAs. A) Schematic of the short and long RAD51C:ATXN7 fusions. In the short isoforms, an aberrant splice causes a premature translation stop codon. The long isoform is in frame and contains all exons. B) Raw sequence of the expressed and cloned short form mRNA. Note that the fusion (splicing exon 7) is shorter than wild-type RAD51C. C) Raw sequence of the expressed and cloned long form mRNA. Note that the fusion (splicing exon 6) is much longer than wild-type RAD51C. The excess sequence is ATXN7.

We screened for RAD51C-ATXN7 fusion mRNA in a panel of 32 breast cancer cell lines. We previously reported that the fusion was present in other cell lines (see previous report), however, using rigorous controls we found out that this was contamination resulting from the very sensitive nested RT-PCR we were using (sequencing revealed the fusion to be identical to the MCF7 fusion consistent with a contamination). Under the new conditions we were not able to find the fusion mRNA in any other cell lines which is consistent with other reports showing that most mRNA fusion products are unique to individual cell lines and tumors (2). The inability to find the fusion mRNA is also consistent with our inability to find the DNA translocation in any other cell lines (see next task).

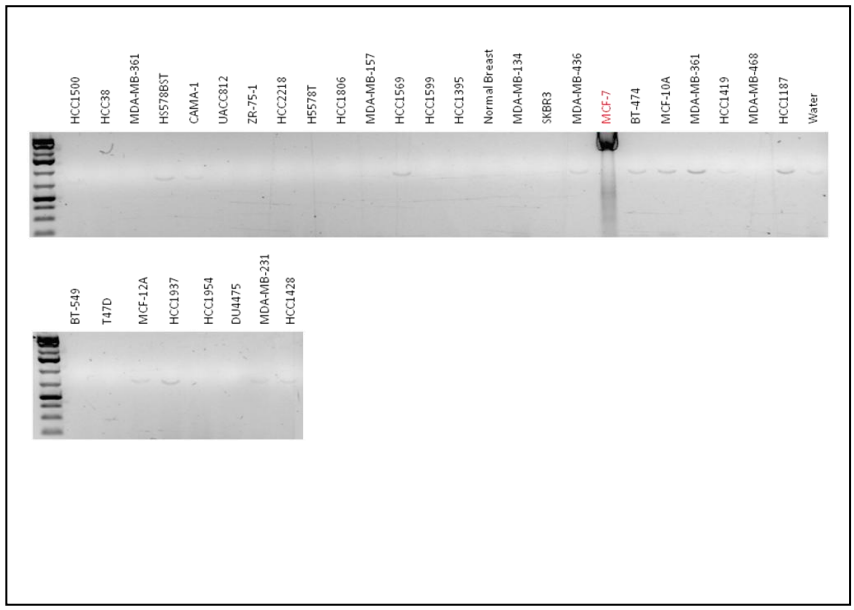

Figure 3: RAD51C:ATXN7 fusion mRNA is only expressed in MCF7 breast cancer cells. RNA was isolated from a panel of 32 breast cancer cell lines and RT-PCR performed using primers in the 5' region of RAD51C and the 3' UTR of ATXN7. Note that a RAD51C:ATXN7 fusion mRNA was only found in MCF7 cells.

The translocations in BRIP1 and EYA2 which we originally reported (1) resulted in truncation of the gene with the final exons being replaced by non-genic DNA. As this eliminates the mRNA polyA tail we believed this would result in an unstable mRNA that is rapidly degraded. Cells with this translocation would thus have one mutant allele and thus would be expected to have either half the level of mRNA compared to normal cells, or if the other allele is also genomically rearranged or mutated they may have no transcript at all. This would be consistent with the classic two-hit hypothesis for tumor suppressor genes. However, to examine this, we needed to directly examine the mRNA produced from specific alleles. To do this, we performed restriction fragment length polymorphism (RFLP) analysis on mRNA isolated from a panel of breast cancer cell lines. For this assay we identified unique single nucleotide polymorphisms (SNPs) in restriction sites (either introducing or deleting a restriction site) that affected restriction enzyme digestion of DNA. We thus amplified BRIP1 and digested the PCR product from a panel of breast cancer cell lines with the specific restriction enzyme to identify cell lines with heterozygous alleles (to allow us to investigate allele specific expression). Figure 4 shows the panel of cell lines and indicates if they have are homozygous for A allele, homozygous for G allele, or are heterozygous with both an A and G. Note that some cells including MCF7 have a ? indicating an imbalance in their alleles as each allele had a band with a different intensity.

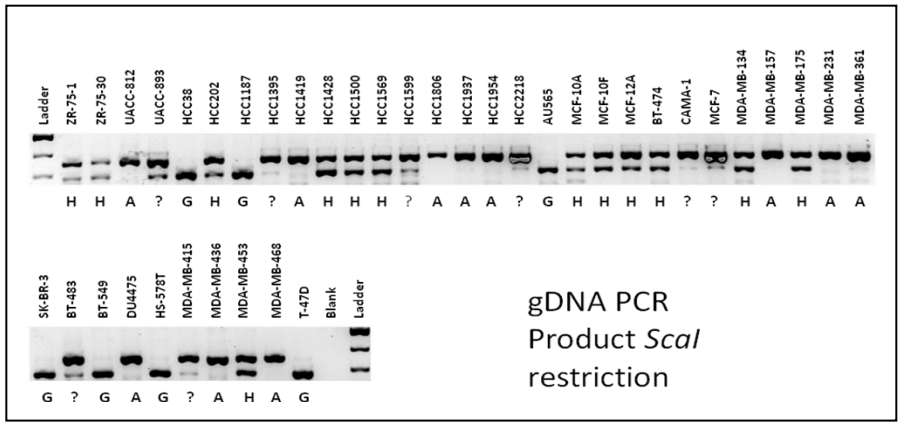

Figure 4: RFLP to examine heterozygosity of BRIP1 in a panel of breast cancer cell lines. PCR for BRIP1 was performed on a panel of breast cancer cell lines. The PCR product was digested with *Sca1* enzyme and visualized by gel electrophoresis. Note that some cells are homozygous (A) for A allele (e.g. UACC-812), homozygous (G) for G allele (e.g. HCC1995), or heterozygous (H) with both A and G (e.g. HCC 1428). Some cell lines showed an imbalance in levels of each allele and are marked with a ? (e.g. MCF7).

Cells should either have one band (homozygous) or two bands (heterozygous) of equal intensity. Some cell lines showed a complex pattern with an imbalance in the alleles (e.g. HCC38, HCC1599). These cell lines were labeled with a ?. Note that MCF-7 cells had an extremely high level of the A allele (so high the black band turned white in the center) and a similar pattern was seen in HCC2218. This suggested that this allele in MCF7 cells maybe amplified, an odd observation for a tumor suppressor gene which should in theory be lost in breast cancer. Supporting this result, a bioinformatic analysis of public databases revealed that BRIP1 is highly amplified in MCF7 cells (consistent with our RFLP analysis) and analysis of data from The Cancer Genome Atlas (TCGA) project showed ~14% amplification of BRIP1 in human breast tumors. This is a highly unusual finding given BRIP1s role as a tumor suppressor gene, and something that was entirely unexpected. We plan on investigating the functional role of this amplification further.

We then performed RT-PCR on cells with were heterozygous for BRIP1 and digested the cDNA product to reveal the relative abundance of mRNA coming from each allele (Figure 5). Consistent with our original observation that one allele of MCF-7 cells has a translocation, and the hypothesis that this results in a null allele, we found allele specific mRNA expression in MCF7 cells. However, note that the expression from this allele is much higher than all other cell lines and significantly higher than MCF10A cells. This would be consistent with the amplification of this allele mentioned above, and a result highly unexpected for a tumor suppressor gene. Note also that all cell lines have mRNA expression higher than MCF10A cells, again a result that is contradictory to BRIP1's role as a tumor suppressor gene. Indeed, as stated previously, this data is consistent with public data and a recent report showing overexpression of BRIP1 in breast cancer.

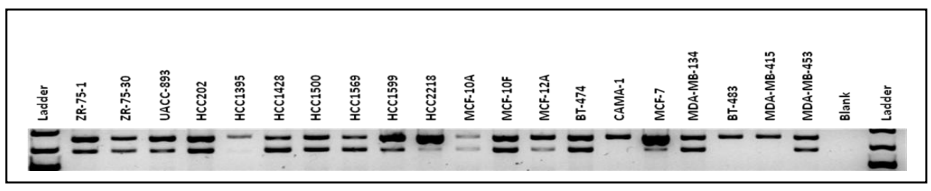

Figure 5: RFLP analysis of mRNA in a panel of breast cancer cell lines. Following RT-PCR, cDNA was digested with Sal1 enzyme and visualized by gel electrophoresis. Note that MCF7 cells have an abundance of mRNA from a single allele. However, also

note that all cell lines have increased mRNA compared to MCF10A immortalized cells, a finding partly inconsistent with BRIP1s proposed role as a tumor suppressor gene.

We took a similar approach to examine allele specific expression of EYA2 in breast cancer cell lines. We first performed restriction digest on genomic DNA with Ear1 restriction enzyme to identify cell lines with heterozygosity. Note that the Ear1 has two restriction sites and so homozygous alleles have two bands while heterozygosity results in 4 bands. Similar to before the cell lines are labeled with their allele type including homozygous A, homozygous C, or heterozygous (H). In contrast to BRIP1, cell lines were clearly identified with each allele type and only MCF-7 cells had discordance indicated with a ?. This is consistent with MCF-7 cells having translocation on one allele and suggests that there are no other rearrangements in EYA2 in the panel of breast cancer cell lines. Of note, ZR-75-30 showed no PCR product indicating likely homozygous deletion at this genomic region.

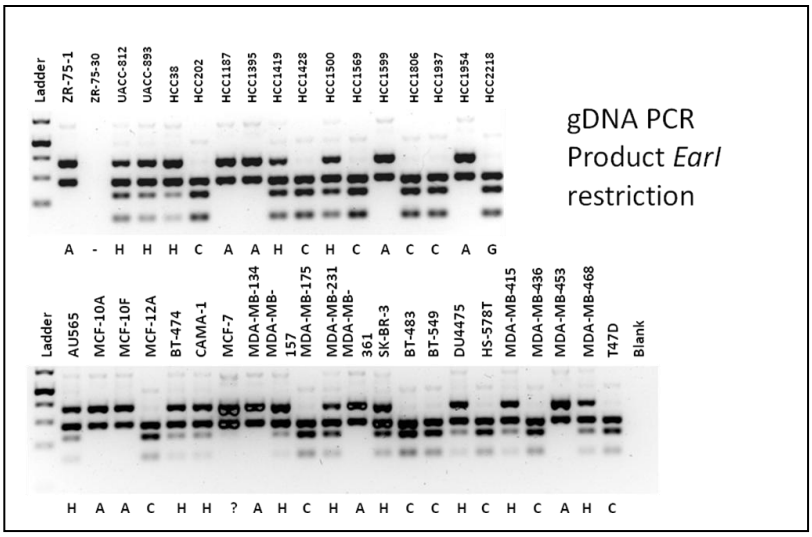

Figure 6: RFLP to examine heterozygosity of EYA2 in a panel of breast cancer cell lines. EYE2 was PCR amplified and DNA digested with Ear1 enzyme and visualized by gel electrophoresis. Note that some cells are homozygous (A) for A allele (e.g. ZR-75-1), homozygous (C) for C allele (e.g. HCC202), or heterozygous (H) with both A and C (e.g. UACC812). Note that ZR-75-30 showed no amplified DNA product suggesting complete loss of this region of DNA.

We then performed RT-PCR on cells with were heterozygous for EYA2 and digested the cDNA product to reveal the relative abundance of mRNA coming from each allele (Figure 7). Consistent with the DNA analysis, MCF-7 cells showed clear allele specific expression of mRNA, with all of the product arising from the A allele. All other cell lines showed heterozygous expression and showed little allelic imbalance.

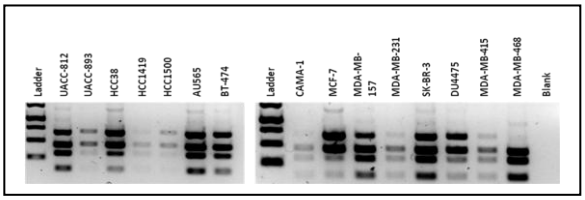

Figure 7: RFLP analysis of EYA2 mRNA in a panel of breast cancer cell lines. Following RT-PCR, EYA2 cDNA was digested with *Ear1* enzyme and visualized by gel electrophoresis. Note that MCF7 cells have an abundance of mRNA from a single A allele. Most other cell lines show heterozygous mRNA expression. Similar to BRIP1, EYA2 is highly expressed in MCF7 cells compared to other cell lines.

3) Measure specific translocations in BRIP1, RAD51C, and EYA2 using PAMP in 20 cell lines. Novel translocations will be identified using Alu or targeted gene walking PCR (months 4-12).

We first confirmed that the BRIP1, RAD51C, and EYA2 DNA translocations were present in different batches of MCF-7 cells (and not in the non-tumorigenic cell line MCF10A) (Figure 8). As noted, PCR of these translocations showed them all to be present.

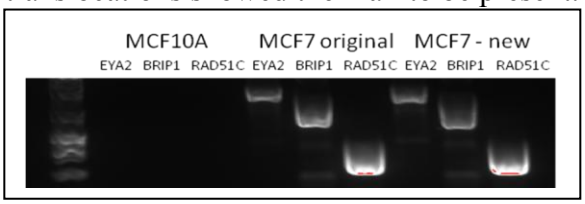

Figure 8: DNA PCR of BRIP1, RAD51C, and EYA2 translocations in two different batches of MCF7, but not MCF10A cells. PCR was performed with primers specific to the translocations of BRIP1, RAD51C, and EYA2 which we previously reported (1). PCR products were visualized by gel electrophoresis.

We next examined whether the translocations were present in a panel of 32 breast cancer cell lines which included MCF-7 cells as a positive control and MCF-10A as a negative control. PCR revealed the translocation in MCF-7 cells but that it was not present in any other cell lines (Figure 9).

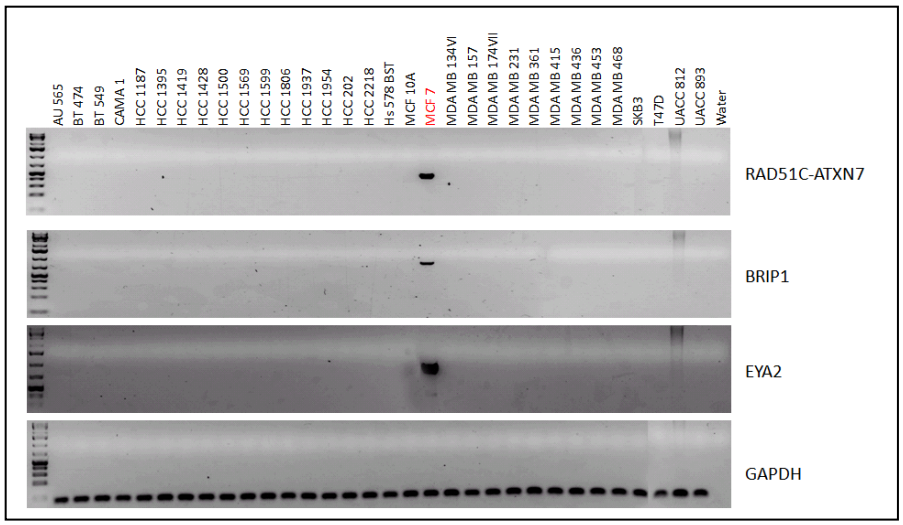

Figure 9: Translocations are only found in MCF-7 breast cancer cells. A panel of 32 breast cancer cell lines were examined for RAD51C, BRIP1 and EYA2 translocations by PCR of genomic DNA. Water served as a negative control for the PCR. Note that the translocations are only found in MCF7 cells.

This suggests that these translocations are private mutations to MCF7 breast cancer cells and not recurrent across other breast cancer cell lines. This data is consistent with other recent reports from the last year showing that DNA translocations (2, 3) and RNA translocations resulting in fusion genes (4) are nearly always private events that exist only in single cell lines or tumors. As we didn't find any novel translocations in any other cell lines we couldn't use Alu or targeted gene walking to identify them.

4) Analyze specific translocations in BRIP1, RAD51C, and EYA2 identified in the cell lines in a pilot study of human breast tumors (n=50) and then a larger definitive set of 200 (months 6-18).

As we didn't find any of the translocations recurrent in any breast cancer cell lines, we didn't examine breast tumors.

5) Measure specific translocations in BRIP1, RAD51C, and EYA2 in a pilot study of 50 breast tumors using break-away fish, and then a larger definitive studying across stages of breast cancer progression (months 12-18).

As we didn't find any of the translocations recurrent in any breast cancer cell lines, we didn't examine breast tumors by FISH.

Aim 2

1) Analyze localization of translocations identified in Aim 1 using fluorescent microscopy of GFP-tagged proteins (months 12-18).

We cloned a myc tag onto the short and long isoforms of RAD51C:ATXN7 to examine expression and localization of the fusion products. While we were able to clone the long isoform, despite repeated attempts we were unable to express the protein. This was attempted with different epitope tags (HA and myc) and by expression in multiple cell lines (HEK293, MCF10A, MCF7). We concluded that the mRNA produced by this long fusion is not suitable for translation. We were however able to clone and express the short isoform (Figure 10). Indeed, immunofluoresence showed the protein (RAD51C:ATXN7 short – right panels) to be expressed at high levels compared to WT RAD51C (left panels). RAD51C:ATXN7 short isoform was expressed in both the cytoplasm and nucleus and localization was similar to myc-tagged wild-type (wt) RAD51C. Irradiation of cells with 2Gy had little effect upon localization of either RAD51C or RAD51C:ATXN7 fusion proteins. As a positive control for RNA damage we observed γ-H2AX foci formation after irradiation (data not shown). This data suggests that the RAD51C short fusion protein doesn't localize differently to wt RAD51C despite the loss of the carboxy terminus.

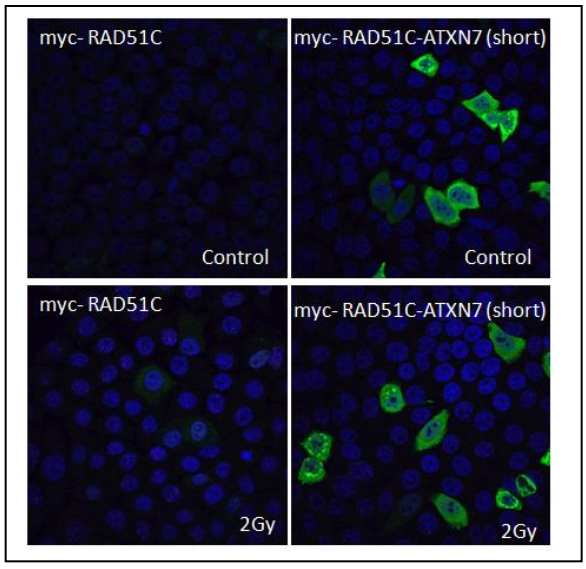

Figure 10: RAD51C:ATXN7 (short isoform) shows cellular localization similar to wild-type RAD51C. Wild type (wt) RAD51C and RAD51C:ATXN7 (short isoform) were tagged with myc and transiently

transfected and expressed in HEK293 cells. The myc tag was visualized by immunofluoresence. The wt RAD51C showed lower expression than the fusion gene, perhaps due to weaker translation or enhanced stability of the fusion mRNA. Note that after irradiation there is little change in the localization of wt or RAD51C:ATXN fusion expression.

2) Test whether the translocation products fail to localize to sites of DNA damage (highlighted by H2AX and RAD51 foci) following irradiation (months 12-18)

As noted in Figure 10 and described above in Aim 2.1, we found that irradiation of cells caused RAD51C and RAD51C:ATXN7 fusion proteins to translocate to the nucleus, but no difference in the localization between these two proteins was noted. This was surprising as the c-terminus of RAD51C is believed to contain domains essential for binding proteins to co-localize with sites of DNA repair.

To examine this further, we expressed RAD51C and RAD51C:ATXN7 (BP86s) as GST-fusion proteins in vitro and tested their ability to bind RAD51B and XRCC3, components of the homologous recombination pathway. RAD51C was found to bind both RAD51B and XRCC3, however, the RAD51C fusion protein (labeled BP86s) was also able to bind with the same affinity. Thus, our hypothesis for that this fusion wouldn't bind to sites of DNA repair due to lack of binding partners in its C-terminus was not correct.

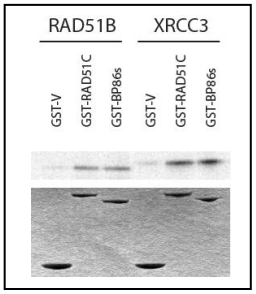

Figure 11: RAD51C:ATXN7 fusion gene binds RAD51B and XRCC3 similar to wild-type RAD51C. We cloned RAD51C and RAD51C:ATXN7 (BP86s) as GST fusion genes and expressed them as *in vitro* proteins (lower panel coomassie stain). GST alone (GST-V) was much smaller than the two fusion genes. Note that GST-BP86s is slightly smaller than GST-RAD51C due to truncation of the c-terminus. In vitro expressed GST fusion proteins were incubated with in vitro expressed RAD51B or XRCC3 and interaction tested via GST pull down assay. Note that GST alone doesn't bind, but both GST-RAD51C and GST-BP86s bind with the same affinity.

3) Test whether expression of the translocations, or a reduction in expression of BRIP1, RAD51C, and EYA2 causes reduced double stranded break repair activity (months 14-20) .

To start this aim we transfected MCF10A cells with a GFP reporter system which is a reporter of double stranded DNA repair activity. The method is shown in Figure 12A. We stably transfected MCF-10A cells with the GFP-reporter plasmid and isolated stable clones which express this plasmid (Figure 12B). However, as we found no data to suggest that any of the three fusion genes resulted in either loss of function, or reduced expression, we didn't use this system to test the effect on DNA repair.

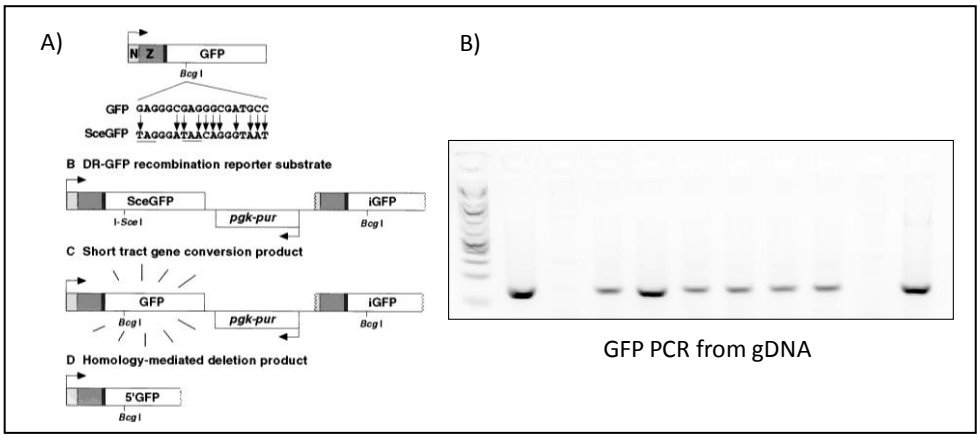

Figure 12: Assay for DNA repair in MCF10A cells: A) Schematic of the GFP reporter assay for measuring DNA repair. B) Stable expression of the GFP reporter plasmid in MCF10A stable clones.

4) Test the effect of the translocations and/or a reduction in expression of BRIP1, RAD51C, and EYA2 on response to DNA damaging agents and other cell biological responses in normal, immortalized and breast cancer cell lines (months 14-24).

Due to the lack of data indicating a role for genomic rearrangements in BRIP1, RAD51C or EYA2 in breast cancer we didn't attempt this aim.

3) Key Research Accomplishments

- MCF-7 cells have a genomic translocation of RAD51C and ATXN7 resulting in the generation of two different mRNA splice isoforms (short and long), the long one containing the N terminus of RAD51C and the full C-terminus of ATXN7
- BRIP1 and EYA2 show allele specific mRNA expression, consistent with translocation of one allele causing a loss of mRNA expression from that allele.
- RAD51C, EYA2 and BRIP1 mRNA expression is higher in MCF-7 and other breast cancer cells than in normal female RNA and MCF-10 cells
- Translocations of RAD51C, EYA2 and BRIP1 are not found in any other breast cancer cell lines
- The short RAD51C:ATXN7 isoform is capable for binding partners for double stranded DNA repair and has cellular localization similar to WT RAD51C

4) Reportable Outcomes

Adrian V. Lee, Petra den Hollander, Oliver A. Hampton, Cristian Coarfa, Aleksandar Milosavljevic. Structural rearrangements in DNA repair genes in human breast cancer. DOD Era of Hope Meeting, Aug 2011, Florida**.**

5) Conclusion

Our hypothesis was that structural genomic alterations in genes that are actually themselves involved in DNA repair enhance the level of genomic instability and ultimately affect breast cancer progression and prognosis. We hypothesized that alterations in *BRIP1*, *RAD51C, and EYA2* would render cell hypersensitive to DNA damaging agents and that fidelity of the DSBR pathway, measured at the genomic level, might be a candidate biomarker for personalizing therapy. However, we found that the translocations we studied are specific to MCF7 cells (private mutations) and not present in any other cell lines. This is consistent with reports published during these studies showing that these translocations are passenger events and not drivers of breast tumorigenesis (3). Further work is required to identify translocations that may be drivers of breast cancer and potential therapeutic targets.

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7) Appendix – Meeting Abstract

Structural rearrangements in DNA repair genes in human breast cancer.

Adrian V. Lee, Petra den Hollander, Oliver A. Hampton, Cristian Coarfa, Aleksandar Milosavljevic.

DOD Era of Hope Meeting, Aug 2011, Florida**.**

The genetic basis of cancer has been firmly established in the last few decades. Genomic instability is a hallmark feature of virtually all breast cancer cells and is caused either by inherited mutations in genes that control genomic fidelity and stability (particularly in DNA repair pathways) or somatic mutations that are acquired during breast cancer progression. The importance of DNA repair in breast cancer is highlighted by the fact that inherited breast cancer is associated with germline mutations in ten different genes associated with genome stability and fidelity. Importantly, the central role of DNA double-strand break repair (DSBR) in both hereditary and sporadic breast cancer may provide an Achilles heel that can be targeted therapeutically. Using new and innovative sequencing methods, we generated a map of breaks in genomic DNA in a breast cancer cell line named MCF-7. This study gave us a unique insight into the genomic instability in MCF-7 cells and showed that a number of genes that had undergone structural change (translocation, deletion, or inversion) were tumor suppressor genes and were mostly repaired by nonhomologous end joining, an error-prone method of DNA DSBR. Intriguingly, we identified translocation of three genes, RAD51C, BRIP1, and EYA2, all of which are all central to DSBR, leading to the novel and exciting IDEA that genes important for genomic integrity and homologous recombination are themselves structurally altered at the genomic level and thus potentially nonfunctional. The RAD51C translocation results in a fusion gene (RAD51C:ATXN7), which was subsequently found expressed in two more breast cancer cell lines. We hypothesize that structural genomic alterations in the genes that are actually themselves involved in DNA repair enhance the level of genomic instability and ultimately affect breast cancer progression and prognosis. Ongoing work is examining the prevalence of recurrent and selected aberrations in BRIP1, RAD51C, and EYA2 in breast cancer cell lines and primary tumors, and we will then test whether truncations or fusions of BRIP1, RAD51C, and EYA2 result in loss of function or dominant negative effects on DNA repair, sensitization to DNA damaging agents, and if the loss of these proteins contributes to genomic instability.